

## PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

Date of mailing (day/month/year)  
01 April 1999 (01.04.99)

To:  
United States Patent and Trademark  
Office  
(Box PCT)  
Crystal Plaza 2  
Washington, DC 20231  
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in its capacity as elected Office

International application No.  
PCT/FI98/00550

Applicant's or agent's file reference  
ÅP2347

International filing date (day/month/year)  
24 June 1998 (24.06.98)

Priority date (day/month/year)  
15 August 1997 (15.08.97)

Applicant

HELLMAN, Jukka et al.

1. The designated Office is hereby notified of its election made:

 in the demand filed with the International Preliminary Examining Authority on:

08 February 1999 (08.02.99)

 in a notice effecting later election filed with the International Bureau on:2. The election  was was not

made before the expiration of 18 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

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## PATENT COOPERATION TREATY

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## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

RECD 08 DEC 1999

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|   |   |   |
|---|---|---|
| Applicant's or agent's file reference<br>ÅP2347   | <b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |   |
| International application No.<br>PCT/FI98/00550   | International filing date ( <i>day/month/year</i> )<br>24 June 1998   | Priority date ( <i>day/month/year</i> )<br>15.08.1997 |
| International Patent Classification (IPC) or national classification and IPC7<br>C 07 K 14/47, C 07 K 16/18, G 01 N 33/68 |   |   |
| Applicant<br>Hellman, Jukka et al   |   |   |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

- I  Basis of the report
- II  Priority
- III  Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV  Lack of unity of invention
- V  Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI  Certain documents cited
- VII  Certain defects in the international application
- VIII  Certain observations on the international application

|  |  |
|--|--|
| Date of submission of the demand<br><br>08.02.1999   | Date of completion of this report<br><br>18.11.1999                                |
| Name and mailing address of the IPEA/SE<br>Patent- och registreringsverket<br>Box 5055<br>S-102 42 STOCKHOLM<br>Facsimile No. 08-667 72 88 | Authorized officer<br><br>Carolina Gómez Lagerlöf/EÖ<br>Telephone No. 08-782 25 00 |

**INTERNATIONAL PRELIMINARY EXAMINATION REPORT**

International application No.

PCT/FI98/00550

**L Basis of the report**

1. This report has been drawn on the basis of (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

 the international application as originally filed. the description, pages 1 - 29, as originally filed,

pages \_\_\_\_\_, filed with the demand,

pages \_\_\_\_\_, filed with the letter of \_\_\_\_\_,

pages \_\_\_\_\_, filed with the letter of \_\_\_\_\_,

 the claims, Nos. \_\_\_\_\_, as originally filed,

Nos. \_\_\_\_\_, as amended under Article 19,

Nos. \_\_\_\_\_, filed with the demand,

Nos. 1 - 13, filed with the letter of 30.08.1999,

Nos. \_\_\_\_\_, filed with the letter of \_\_\_\_\_,

 the drawings, sheets/fig 1 - 8, as originally filed,

sheets/fig \_\_\_\_\_, filed with the demand

sheets/fig \_\_\_\_\_, filed with the letter of \_\_\_\_\_,

sheets/fig \_\_\_\_\_, filed with the letter of \_\_\_\_\_,

2. The amendments have resulted in the cancellation of:

 the description, pages \_\_\_\_\_ the claims, Nos. \_\_\_\_\_ the drawings, sheets/fig \_\_\_\_\_

3.  This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

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**III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

 the entire international application, claims Nos. 12-13

because:

 the said international application, or the said claims Nos. 12-13relate to the following subject matter which does not require an international preliminary examination (*specify*):

See PCT Rule 67.1.(iv).: Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

 the description, claims or drawings (*indicate particular elements below*) or said claims Nos.are so unclear that no meaningful opinion could be formed (*specify*): the claims, or said claims Nos. \_\_\_\_\_ are so inadequately supported  
by the description that no meaningful opinion could be formed. no international search report has been established for said claims Nos. \_\_\_\_\_

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**V. Resoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement****1. Statement**

|                               |        |             |     |
|-------------------------------|--------|-------------|-----|
| Novelty (N)                   | Claims | <u>1-11</u> | YES |
|                               | Claims | _____       | NO  |
| Inventive step (IS)           | Claims | <u>1-11</u> | YES |
|                               | Claims | _____       | NO  |
| Industrial applicability (IA) | Claims | <u>1-11</u> | YES |
|                               | Claims | _____       | NO  |

**2. Citations and explanations**

The claims disclose isolated osteocalcin (hOC) fragments from human urine, monoclonal or recombinant antibodies with the capability of binding the osteocalcin fragments and an immunoassay for quantitative determination of the fragments.

During the search the following documents were found:

A EP, A1, 557663

B Journal of Bone and Mineral Research, Vol. 11, No 8, 1996,  
1165-1175

C Peptide Research, Vol. 7, No 4, 1994, 171-174

Document A discloses a method for the assessment of bone fragility and osteoporosis fracture risk by measuring in vitro the concentration of under-carboxylated osteocalcin in a biological fluid sample such as serum, plasma or urine. Serum is particularly preferred. Monoclonal antibodies that recognise the under-carboxylated osteocalcin are also claimed. Under-carboxylated osteocalcin signifies osteocalcin that has 0-2 gamma-carboxylations instead of 3 as in the normal osteocalcin. The document states that it is difficult to isolate sufficient amounts of under-carboxylated osteocalcin and that it is preferable to use recombinant or synthetic under-carboxylated osteocalcin.

Claim 1 in the application covers both the normal osteocalcin and under-carboxylated osteocalcins. The difference is that the claimed fragment is isolated from human urine. The prior art does not disclose how to isolate under-carboxylated osteocalcin from urine and nor is its exact structure previously known.

It is an advantage to use urine samples since there is great diurnal variations in the serum concentration hOC. Further it is not known in the art to use non-competitive immunometric determination of urine derived hOC.

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**Supplemental Box**

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

Document B relates to different monoclonal antibodies, which binds to osteocalcins. In the discussion (pp 1172-1174) it is mentioned that there are different forms of osteocalcin in circulation in the body fluids. Different fragments of osteocalcin are shown.

Document C shows that gamma-carboxylation in position 17 is essential for a calcium-dependent conformational transition.

Documents A, B and C show the general state of the art.

Thus, claims 1-11 are considered to fulfil the requirements of novelty, inventive step and industrial applicability.

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**VI. Certain documents cited****1. Certain published documents (Rule 70.10)**

| Application No.<br>Patent No. | Publication date<br>(day/month/year) | Filing date<br>(day/month/year) | Priority date (valid claim)<br>(day/month/year) |
|-------------------------------|--------------------------------------|---------------------------------|---|
| EP 834740                     | 16.10.1997                           | 08.04.1998                      | 10.04.1996                                      |

**2. Non-written disclosures (Rule 70.9)**

| Kind of non-written disclosure | Date of non-written disclosure<br>(day/month/year) | Date of written disclosure<br>referring to non-written disclosure<br>(day/month/year) |
|--------------------------------|--|---|
| _____                          | _____  | _____   |

## CLAIMS

1. An isolated osteocalcin fragment derived from human urine, said fragment characterized in that at least one of the glutamic acids in the position 17, 21 and 24  
5 of the amino acid sequence

6      7

Tyr-Leu-Tyr-Gln-Trp-Leu-Gly-Ala-

Pro-Val-Pro-Tyr-Pro-Asp-Pro-Leu-

17                          21                          24

Glu-Pro-Arg-Arg-Glu-Val-Cys-Glu-Le-

30

Asn-Pro-Asp-Cys-Asp-Glu-Leu-Ala-

Asp-His-Ile-Gly-Phe-Gln-Glu-Ala-

Tyr-Arg-Arg-Phe-Tyr-Gly-Pro-Val

is gamma-carboxylated.

2. The fragment according to claim 1 characterized in that the fragment spans from  
25 the amino acid in position 7 to the amino acid in position 30 of the amino acid  
sequence described in claim 1, and that all three glutamic acids in the positions 17,  
21 and 24 of said sequence are gamma-carboxylated.

3. The fragment according to claim 1 characterized in that the fragment spans from  
30 the amino acid in position 6 to the amino acid in position 30 of the amino acid  
sequence described in claim 1, and that all three glutamic acids in the positions 17,  
21 and 24 of said sequence are gamma-carboxylated.

4. A monoclonal antibody or recombinant antibody fragment characterized by the capability of binding the human gamma-carboxylated osteocalcin fragment according to claim 1, 2 or 3.
5. The monoclonal antibody or recombinant antibody fragment according to claim 4 characterized by the specificity to epitopes that have been identified on the gamma-carboxylated fragment of osteocalcin, wherein said fragment spans either
  - i) from the amino acid in position 7 to the amino acid in position 30, or
  - ii) from the amino acid in position 6 to the amino acid in position 30
- 10 of the amino acid sequence described in claim 1, and that all three glutamic acids in the positions 17, 21 and 24 of said sequence are gamma-carboxylated.
6. A cell line producing the monoclonal antibody according to claim 4 or 5.
- 15 7. An immunoassay for quantitative determination of a gamma-carboxylated osteocalcin fragment according to claim 1 characterized in that a sample containing said fragment is exposed to a monoclonal antibody or recombinant antibody fragment which binds said gamma-carboxylated osteocalcin fragment.
- 20 8. The immunoassay according to claim 7 characterized by employing a monoclonal antibody or recombinant antibody fragment specific to epitopes that have been identified on the gamma-carboxylated fragment of osteocalcin, wherein said fragment spans either
  - i) from the amino acid in position 7 to the amino acid in position 30, or
  - 25 ii) from the amino acid in position 6 to the amino acid in position 30
- of the amino acid sequence described in claim 1, and that all three glutamic acids in the positions 17, 21 and 24 of said sequence are gamma-carboxylated.

9. The immunoassay according to claim 7 or 8 characterized in that the immunoassay is non-competitive employing at least two different monoclonal antibodies or recombinant antibody fragments.
- 5      10. The immunoassay according to claim 9 characterized in that the non-competitive immunoassay is carried out in either a one-step or a two-step incubation procedure.
- 10     11. The immunoassay according to claim 9 characterized in that the two monoclonal antibodies employed are the antibodies 2H9 and 6F9 that recognize the C-terminal and N-terminal epitopes on the fragment which was determined to be 3005.
12. The immunoassay according to claim 9 characterized in that the two monoclonal antibodies employed are the antibodies 6F9 and 1C4 that recognize the N-terminal and the C-terminal epitopes on the measured osteocalcin fragments (6-30 or 7-30).
- 15     13. The immunoassay according to claim 9 characterized in that the two monoclonal antibodies employed are the antibodies 6F9 and 3H8 that recognize the N-terminal and the C-terminal epitopes on the measured osteocalcin fragments (6-30 or 7-30).
- 20     14. A method for the measurement of the rate of bone turnover (formation and/or resorption) and/or for the investigation of metabolic bone disorders in an individual, characterized by quantitative determination of a fragment according to any of the claims 1 to 3.
- 25     15. The method according to claim 14 characterized in that an immunoassay according to any of the claims 7 - 13 is employed.